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ANALYSIS OF HEMOLYMPH OXYGEN LEVELS AND ACID-BASE STATUS DURING EMERSION '*IN SITU*' IN THE RED ROCK CRAB, *CANCER PRODUCTUS*[1]

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ABSTRACT

Hemolymph samples were taken from small (<100 g) individuals of *Cancer productus* following *ca.* 3 h air exposure (emersion) on the beach, '*in situ*', at Friday Harbor, Washington. Compared with crabs of similar size in sea water in the laboratory, these crabs emersed '*in situ*' had lower Pa_{O_2}, and Pv_{O_2}, but no significant change in pH and a small, not significant, internal hypercapnia. Total CO_2 (C_{CO_2}) content of the hemolymph was elevated by 70% (15.2 *versus* 9.0 m*M*), possibly as compensation for input of acid into the hemolymph. These responses are qualitatively similar to those resulting from similar treatment in the laboratory, but differ in the reduced magnitude of the internal hypercapnia and acidosis of the hemolymph. It is suggested that the particular conditions of emersion '*in situ*' permit some gas exchange with interstitial sea water. Interstitial sea water was found to be hypoxic (P_{O_2} = 20–40 torr), which would limit oxygen supply yet permit CO_2 excretion to continue, in agreement with the data.

INTRODUCTION

Intertidal decapod crustaceans may face exposure to air and hence the transition from aquatic to aerial respiration on a daily or more frequent basis, depending on tide cycles and amplitudes. Reasonably complete patterns of respiratory responses during short term air exposure (emersion) have been described for two marine crabs, *Carcinus maenas* (Truchot, 1975; Taylor and Butler, 1978) and *Cancer productus* (deFur and McMahon, 1984a, b) and also for freshwater crayfish, *Austropotamobius pallipes* (Taylor and Wheatly, 1980). Less complete patterns of response to emersion have also been described for several other marine crabs (McDonald, 1977; O'Mahoney, 1977; Batterton and Cameron, 1978). These laboratory studies indicate that short term emersion is associated with an acidosis, which may be respiratory, as in *Carcinus maenas* (Truchot, 1975; Taylor and Butler, 1978) or mixed respiratory and metabolic as in *Austropotambius pallipes* (Taylor and Wheatly, 1980) and *Cancer productus* (deFur and McMahon, 1984b). Compensation for the acidosis in all species studied occurs largely via a rise of hemolymph bicarbonate, although the process is still incomplete in 3–4 h. Gas exchange during emersion is probably diffusion limited in all species studied and may also be perfusion limited in some species (deFur and McMahon, 1984a). All of these studies, however, have been conducted in the laboratory

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and it is not known how closely these 'laboratory' responses mimic those occurring '*in situ*' under natural conditions.

The present study, therefore, describes hemolymph acid-base conditions and oxygen levels in the crab, *Cancer productus* Randall during '*in situ*' emersion at low tide on the beach. These hemolymph samples provide respiratory data obtained from crabs air exposed in their natural habitat and unaffected by previous laboratory manipulations.

Materials and Methods

This study was undertaken at the Friday Harbor Laboratories of the University of Washington on San Juan Island, Washington. Hemolymph was sampled while animals were air exposed on the beach '*in situ*' at low tide. Animals from the same vicinity were also collected and maintained in flowing natural sea water at the Friday Harbor Laboratories to provide comparative data from immersed crabs. All crabs held in the laboratory were kept at ambient sea water temperature (9–10°C) in darkened aquaria provided with 5–10 cm of fine sand and gravel substrate. These crabs were fed 2–3 times weekly except within 24 h of experiments.

Initially, an intertidal area where individuals were routinely emersed at low tide was located, the time of exposure noted, and approximately 3 h later, hemolymph samples were taken. Crabs were usually buried in the substrate beneath rocks or kelp; hence it was necessary first to lift the kelp or a rock, locate a crab, and then rapidly take the hemolymph samples. All hemolymph samples were withdrawn into iced, 1 ml glass syringes, which were immediately sealed and replaced on ice. Postbranchial (arterial) samples were taken by carefully puncturing the dorsal carapace, anteriolateral to the heart using the syringe needle, and then withdrawing 0.2–0.4 ml of hemolymph. Prebranchial (venous) samples were taken from the base of the fifth walking leg by gently restraining the crab and lifting the posterior end partially out of the substrate. The iced samples were then returned to the laboratory for analysis. Burnett and Bridges (1981) report that sealed, ice hemolymph samples may be kept for at least 1 h with no significant changes in acid-base or O_2 variables. This conclusion was tested and verified using three samples in the present study.

Postbranchial samples could be obtained quickly and with a minimum of disturbance to the animals because the crabs remained motionless in the substrate. However, partial removal from the substrate, as was necessary during prebranchial sampling, or repeated prodding always provoked evasive behavior. Thus, if a prebranchial sample could not be obtained swiftly on the first attempt, the sample was discarded. Both postbranchial and prebranchial samples were obtained sequentially from 8 crabs fully emersed '*in situ*', and these were treated statistically as paired samples.

Hemolymph samples were analyzed for pH, total CO_2 (C_{CO_2}), CO_2 tension (P_{CO_2}), and O_2 tension (P_{O_2}), although small sample volume frequently prohibited making all measurements on each sample. Hemolymph pH was measured with a Radiometer capillary electrode (G299A) thermostatted to 9–10°C and connected to an acid-base analyzer (Radiomenter PHM 71). C_{CO_2} was determined on 40 μl of hemolymph using the method of Cameron (1971) with each sample measurement preceded and followed by 15 μl standard injections of 30 m*M* $NaHCO_3$. Hemolymph P_{CO_2} was measured using a Radiomenter electrode (E 5036-0) thermostatted to 9–10°C and the signal displayed on an acid-base analyzer (Radiometer PHM 71) set to 10× sensitivity. The electrode was calibrated with humidified gases of known P_{CO_2} delivered via a Wosthoff pump. Measures of P_{O_2} were made with a Radiometer

electrode (E 5047), thermostatted to 10°C, and an acid-base analyzer (Radiometer PHM 71).

Statistical analyses

Statistical analyses were performed using Student's *t*-test for either grouped or paired variates and the 0.05 level was used as the criterion of significance. Regressions were performed via the least squares estimation. Mean values ($\bar{x}$) in the text are given ± one standard error (S.E.).

RESULTS

General observations

Individuals of *Cancer productus* were abundant in shallow water (<1.5 m) in the vicinity of the Friday Harbor Laboratories during November, 1979, but large numbers of crabs were found air exposed at low tide only in Beaverton Cove. This particular area was protected, permitting the growth of a large kelp bed which covered the lower intertidal zone during low tides. The substrate was predominantly coarse sand mixed with fine gravel and restricted areas of loose, fine gravel. During low tide, *C. productus* were most often found buried in the substrate beneath rocks or kelp with only the most anterior-dorsal aspect of the shell protruding above the sand. Crabs were observed emersed on top of the substrate only once and all but one of these were beneath thick layer of kelp. On several occasions, crabs were found buried in fine substrate which still held noticeable amounts of interstitial sea water. Postbranchial hemolymph samples were obtained from 7 of these crabs and 4 samples of the interstitial water were also obtained for measurement of P_{O_2}.

The mean weight of 30 crabs, which were not sampled but collected and returned to the laboratory was 29.63 ± 2.40 g. Data from these animals were used to describe the relationship between wet weight and carapace width (Fig. 1), from which the mean wet weight of crabs sampled '*in situ*' was estimated to be 21.02 ± 2.16 g. It is interesting to note that there is a semilogarithmic relationship between carapace width and wet weight. Tides which were sufficiently low to result in emersion of *C. productus*

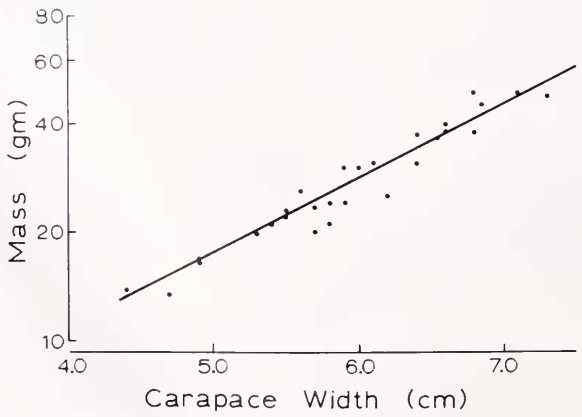

FIGURE 1. Semilogarithmic relationship between body mass and carapace width in *C. productus*, used to calculate wet weight of crabs sampled *in situ*. r = 0.99.

occurred after dark, therefore air temperatures during sampling did not differ significantly from the sea water temperatures of 9–10°C.

Crabs immersed in the laboratory

Mean values of P_{O_2}, pH, C_{CO_2}, and P_{CO_2} in pre- and postbranchial hemolymph of immersed crabs held in the laboratory for 3–10 days are given in Table I. Pa_{O_2} and Pv_{O_2} were typical of values reported previously for small *C. productus* exhibiting primarily unilateral ventilation (deFur and McMahon, 1984a). The acid-base system of immersed crabs in the laboratory was characterized by a high pH, low P_{CO_2}, and low C_{CO_2} (Table I). These values and P_{O_2} levels of crabs immersed in the laboratory were used as a baseline with which to compare emersed crabs *in situ*.

Hemolymph samples were obtained from 22 small *C. productus* which had been emersed on the beach, *in situ* for approximately 3 h. Pa_{O_2} and Pv_{O_2} were significantly lower in crabs emersed *in situ* than in immersed crabs, yet there remained a significant difference between Pa_{O_2} and Pv_{O_2} of 6.85 torr (Table I). This difference was the same regardless of whether the data were analyzed as grouped or as paired date, *i.e.*, using samples taken sequentially from the same crabs (Table I). Hemolymph O_2 content was not measured in the present study, yet the amount of oxygen delivered to the tissues in crabs emersed *in situ* can be estimated using oxygen equilibrium curves determined by deFur and McMahon (1984a) for hemolymph from crabs of similar size at 10°C, 34‰ (Fig. 2). In spite of the low *in vivo* P_{O_2}'s measured during *in situ* emersion, hemocyanin was more than 70% oxygen saturated in transit through the gills, and only 12% oxygen saturated in hemolymph returning from the tissues (Fig. 2). At a mean hemolymph oxygen carrying capacity of 0.466 m*M* (deFur and McMahon, 1984a), this represents 0.141 mmol O_2 per liter of hemolymph delivered to the tissues. Unloading of oxygen from hemocyanin at the tissues was enhanced by approximately 20% via a normal Bohr shift (see below).

TABLE I

Hemolymph oxygen tensions and acid base status of C. productus *during emersion* in situ *and in interstitial water*

	P_{O_2} (torr)	pH	C_{CO_2} (m*M*)	P_{CO_2} (torr)
Emersed *in situ*				
postbranchial	12.38 ± 1.35 (16)	7.948 ± 0.023 (16)	15.23 ± 0.67 (15)	2.50 ± 0.22 (12)
prebranchial	5.85 ± 1.05 (6)	7.906 ± 0.031 (13)	15.96 ± 0.92 (13)	2.82 ± 0.29 (5)
pre-postbranchial (paired)[1]	6.3 ± 1.9 (6)	0.072 ± 0.020 (8)	1.78 ± 0.47 (8)	0.267 ± 0.09 (5)
Interstitial				
In situ	16.86 ± 2.32 (7)	7.899 ± 0.055 (7)	15.89 ± 0.74 (7)	2.29 ± 0.21 (6)
Immersed in laboratory				
postbranchial	58.85 ± 10.7 (13)	7.960 ± 0.03 (12)	8.95 ± 0.75 (7)	1.97 ± 0.31* (7)
prebranchial	19.04 ± 3.0 (14)	7.921 ± 0.033 (13)	—	—

Mean ± S.E. (N).

[1] Paired samples were taken sequentially and the data analyzed as paired variates.

* Calculated using the method of Wilkes *et al.* (1980).

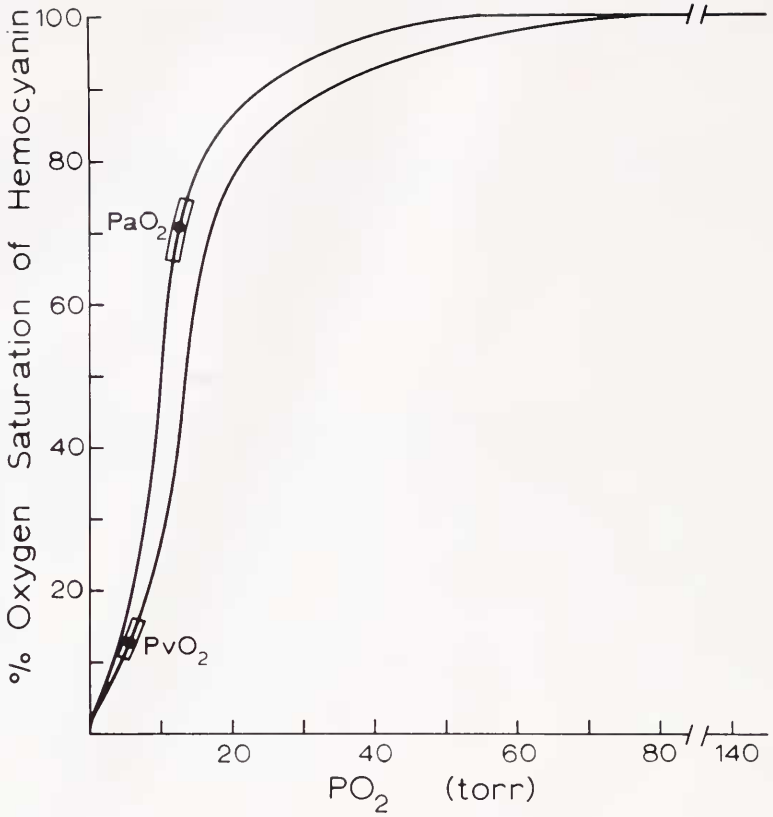

FIGURE 2. Oxygen binding curves for hemocyanin of small *C. productus* at pHa = 7.98 and pHv = 7.90 using data from deFur and McMahon (1984a) and deFur (1980).

Although hemolymph pH during *in situ* emersion was not significantly different from corresponding values in immersed crabs (Table 1) other variables in the acid-base system of hemolymph in crabs emersed *in situ* were nonetheless dissimilar. Pa_{CO_2} was slightly (not significantly) higher in emersed crabs '*in situ*', but Ca_{CO_2} was significantly (70%) higher ($P < .05$) (Table I), indicating a large base excess. Sequential samples of pre- and postbranchial hemolymph from these naturally emersed crabs exhibited significant differences between the mean values of all three acid-base variables (paired observations). Pv_{CO_2} and Cv_{CO_2} were higher and pHv lower than the corresponding values for postbranchial hemolymph, indicating that branchial excretion of CO_2 continued during emersion (deFur and McMahon, 1984b).

Postbranchial hemolymph samples were also taken from seven crabs emersed *in situ* but buried in substrate containing obvious interstitial sea water. These crabs were clearly able to circulate some of this water through the branchial chambers since water could often be seen flowing from the exhalant branchial apertures. Acid-base conditions of hemolymph in these animals were, however, not significantly different from crabs emersed in adjacent but drier areas, although Pa_{CO_2} was slightly ($P > .05$) lower. Mean Pa_{O_2} of the crabs obviously utilizing interstitial sea water was only 4.5 torr higher ($P > .05$) than in those from drier areas, but was significantly reduced from that of immersed crabs. This low mean Pa_{O_2} was likely a consequence of the hypoxic nature of the interstitial water ($P_{O_2} = 27 \pm 4.5$ torr; n = 4).

The responses of small *C. productus* to emersion in substrate containing interstitial water was further investigated in the laboratory. Ambient P_{O_2} fell from 150 torr to 51 torr in the first hour and decreased further to 31 torr by 4 h. Hemolymph Pa_{O_2} fell rapidly during initial exposure (Fig. 3), and continued to decline slowly; mean Pa_{O_2} over the 0.75–4.0 h period was only 14.3 ± 1.5 torr. Mean Pa_{O_2} of samples taken from these crabs was not significantly different from mean Pa_{O_2} of either group of crabs sampled *in situ* on the beach. Hemolymph pHa of crabs in interstitial water in the laboratory was quite variable (Fig. 3) and the mean was not significantly different from that of any of the groups of crabs sampled on the beach. Hemolymph Ca_{CO_2} of crabs exposed to interstitial water in the laboratory increased linearly during 4 h (Fig. 3), reaching levels similar to those in crabs emersed *in situ*.

Discussion

The data obtained at Friday Harbor for crabs immersed in flowing natural sea water, at sea level, in the laboratory at 9–10°C and 34‰ salinity, compare well with those obtained at similar temperature and salinity in a recirculating sea water system at an altitude of 1050 m in Calgary (Table II). Pa_{O_2}, Pa_{CO_2}, and Ca_{CO_2} were slightly

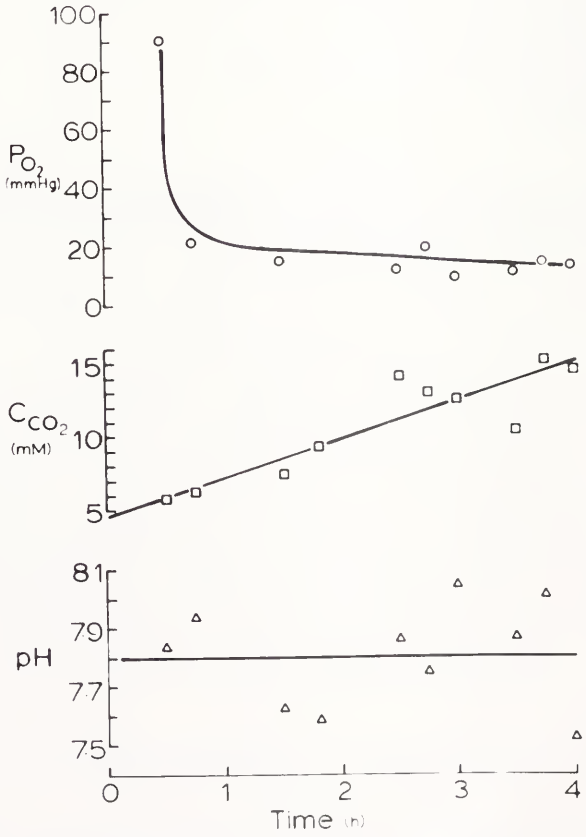

FIGURE 3. Postbranchial hemolymph P_{O_2}, C_{CO_2}, and pH in individual small *C. productus* emersed for 4 h in substrate containing interstitial sea water in the laboratory. Line fitted by eye for P_{O_2}, by least squares estimation for C_{CO_2} ($r = 0.99$), and through $\bar{x}$ for pH. Symbols represent individual values.

TABLE II

Hemolymph P_{O_2} and acid-base status of small C. productus *immersed in sea water (10°C, 32–35‰ salinity) and the changes resulting from 3–4 h emersion in the laboratory and* in situ

Location	Immersed crabs			
	Pa_{O_2} (torr)	pHa	Ca_{CO_2} (m*M*)	Pa_{CO_2} (torr)
Calgary[1]	50.7 ± 8.0 (8)	8.017 ± 0.02 (10)	7.31 ± 0.44 (7)	1.33 ± 0.05 (7)
Friday Harbor[2]	58.9 ± 11 (11)	7.960 ± 0.03 (12)	8.95 ± 0.75 (7)	1.97 ± 0.31* (7)

	Changes during emersion					
	ΔPa_{O_2} (torr)	ΔPv_{O_2} (torr)	ΔpHa	ΔPa_{CO_2} (torr)	ΔC_{CO_2} (m*M*)	pHa–pHv
In situ[2] (3–4 h) (Friday Harbor)	−46.5	−13.2	−0.012	+0.53	+6.28	0.072
Laboratory[1] (4 h) (Calgary)	−37.2	−10.7	−0.147	+2.27	+8.72	0.034

[1] deFur and McMahon, 1984b.
[2] Table I.
* Calculated using the method of Wilkes *et al.* (1980).
Data are $\bar{x}$ + 1 S.E. (n).

higher and pHa slightly lower in Friday Harbor than in Calgary as might be expected from the change in altitude, but none of these differences was significant. deFur and McMahon (1984a) also observed similar respiratory behavior patterns in immersed *C. productus* regardless of location. These observations indicate that the respiratory status of *C. productus* is affected little by the differences between experimental conditions in Calgary and those more similar to the natural habitat.

The present data are the first hemolymph acid-base status or oxygen tensions reported for decapods *in situ* during air exposure. A greater degree of variability than usually occurs in laboratory studies was noted in some variables, perhaps because factors such as nutritional state and molting stage are not controlled, as under laboratory conditions. An important aspect of the present study is the qualitative similarity between the responses of small *C. productus* to emersion on the beach in Friday Harbor and in the laboratory in Calgary (Table II); under both experimental regimes P_{O_2} and pH decreased, and C_{CO_2} and P_{CO_2} increased. The decreases in both Pa_{O_2} and Pv_{O_2} were greater under natural conditions than in the laboratory, but these differences between responses *in situ* and in the laboratory are not significantly different. Additionally, under both conditions, hemocyanin is well oxygenated at the gill and most of the O_2 is removed in passage through the tissues (Fig. 2 and deFur and McMahon, 1984a).

Crabs emersed under laboratory conditions (deFur and McMahon, 1984b) exhibited a marked acidosis due in part to a significant increase in P_{CO_2}. In contrast, crabs emersed '*in situ*' showed neither a significant acidosis nor increase in P_{CO_2}. The small decrease in pH in these crabs (Table I) was less, however, than would be expected on the basis of the *in vitro* buffering properties (deFur and McMahon, 1984b), suggesting that more effective compensation occurred '*in situ*'. The more than 6 m*M* increase of C_{CO_2} implies that there is some net input of acid which is compensated by elevation of HCO_3^-. The relative contribution of other acids, especially metabolic ones such

as lactic acid, to the acid-base status of crabs emersed '*in situ*' is not known. Thus, the present study cannot identify with certainty the compensatory mechanisms involved. However, the greater pHa–pHv difference and lower Pa_{CO_2} measured in crabs emersed '*in situ*' suggest that CO_2 excretion may be more effective under these conditions.

Maintenance of branchial CO_2 excretion implies maintained ventilation and perfusion of the gills during emersion. deFur and McMahon (1984a) measured maintained sub-ambient branchial pressures in small *C. productus* during emersion in the laboratory, and reasoned that interstitial sea water could be aspirated into the branchial chamber. This water could allow CO_2 excretion to continue during emersion but seems to have no effect on O_2 uptake since Pa_{O_2} is depressed (Table I). This situation is not paradoxical since a) CO_2 diffuses more effectively in aqueous systems, and b) interstitial sea water samples, though more highly oxygenated than finer sediments, were still hypoxic. Thus, irrigation of the gills with interstitial sea water could allow CO_2 excretion with little effective oxygenation. Under the laboratory conditions used by deFur and McMahon (1984a, b), care was taken to remove as much sea water, including interstitial, as possible, precluding its use for branchial functions.

The observed acid-base changes during emersion *in situ* show a discrepancy between measured and calculated P_{CO_2} similar to that observed in the laboratory (deFur, Wilkes and McMahon, 1980). This discrepancy is clearly apparent on a "Davenport diagram" (Fig. 4) and precludes use of such a diagram for analysis of the acid-base system. A discrepancy occurs only during emersion and was associated with large, rapid elevations of hemolymph C_{CO_2}, indicating dynamic rather than steady-state conditions. As noted by deFur *et al.* (1980), data from crabs immersed in sea water are described perfectly on the Davenport diagram.

In a similar study, Toulmond (1973) described the responses of the intertidal polychaete *Arenicola marina* during 4 h emersion '*in situ*'. *Arenicola* also experienced a decrease of Pv_{O_2}, nearly exhausting the otherwise substantial venous oxygen reserve.

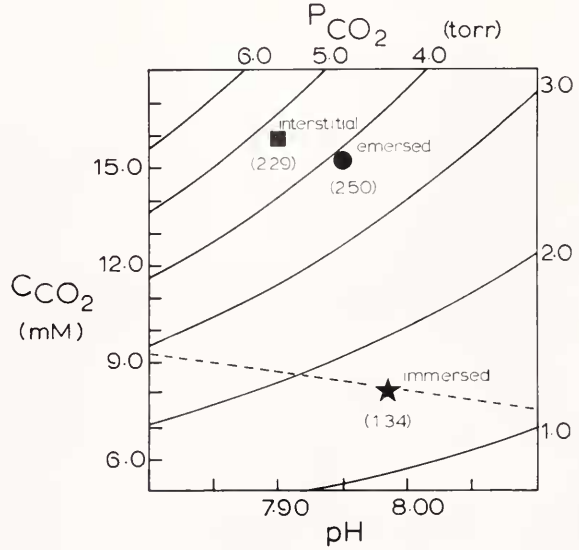

FIGURE 4 "Davenport diagram" relating C_{CO_2}, pH, and P_{CO_2} in the hemolymph of *C. productus* according to the method of Wilkes *et al.* (1980). The diagonal line (– – –) represents the *in vitro* buffer capacity. Points depict mean *in vivo* values from Table I with measured P_{CO_2} given in (□) beneath the symbol.

Simultaneously, there was an internal hypercapnia with a subsequent acidosis (respiratory) and rise of blood bicarbonate (Toulmond, 1973). This author concludes that gas exchange is impaired under these conditions and anaerobiosis occurs, contributing a metabolic component to the acidosis. The responses of small *C. productus* under similar conditions (Table I) are qualitatively similar to those of *Arenicola,* but are quantitatively quite different. The decrease in P_{O_2} and pH and the increase in P_{CO_2} are less in small *C. productus.* These differences are likely due to some air breathing capability of the crabs, and availability and utilization of sea water during emersion. *Arenicola marina* ceases all ventilation, normally accomplished by body movements forcing water through the burrow. Small *C. productus,* however, are able to utilize the hypoxic interstitial sea water, permitting CO_2 excretion but limiting oxygen supply.

Small *C. productus* occupy a restricted habitat within the intertidal zone and during air exposure remain buried in the substrate in locations where sea water drains from the substrate relatively slowly. In this condition, the small crabs can maintain acid-base balance for the few hours of emersion, yet must endure a reduction in oxygen supply. Thus, these small crabs which have access to interstitial water may not be able to maintain oxygen uptake in air, but do not have the problem of carbon dioxide excretion which is the major respiratory problem of truly intertidal crabs and true air breathers.

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